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Oregon State Univ., Corvallis

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PERSISTENCE AND DETECTION OF COLIFORMS IN TURBID FINISHED DRINKING WATER

by

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Cooperative Agreement CR806287

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16. ABSTRACT

To define interrelationships between elevated turbidities and the efficiency of chlorination in drinking water, experiments were conducted to measure bacterial survival, chlorine demand, and interference with microbiological determinations. Results indicated that disinfection efficiency (log-fold decrease in coliform numbers) was negatively correlated with turbidity and was influenced by season, chlorine demand of the samples, and the initial coliform level. Interference with coliform detection in turbid water could be demonstrated by the recovery of typical coliforms from apparently negative filters.

A procedure was developed to detect false negative reactions (interference) in the standard most probable number (S-MPN) technique for coliform enumeration of potable water supplies.

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FOREWORD

The U.S. Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimonies to the deterioration of our natural environment. The complexity of that environment and the interplay of its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution; it involves defining the problem, measuring its impact, and searching for solutions. The Municipal Environmental Research Laboratory develops new and improved technology and systems to prevent, treat, and manage wastewater and solid and hazardous waste pollutant discharges from municipal and community sources, to preserve and treat public drinking water supplies, and to minimize the adverse economic, social, health, and aesthetic effects of pollution. This publication is one of the products of that research and provides a most vital communications link between the researcher and the user community.

This report presents the results of an investigation which examined the impact of turbidity on chlorine disinfection and the persistence of coliform bacteria in finished drinking water supplies. In addition, the influence of turbidity and high numbers of non-coliform bacteria on the detection of coliforms in drinking water is investigated.

Francis T. Mayo, Director
Municipal Environmental Research
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ABSTRACT

To define interrelationships between elevated turbidities and the efficiency of chlorination in drinking water, experiments were conducted to measure bacterial survival, chlorine demand, and interference with microbiological determinations. Experiments were conducted on the surface water supplies for communities that practice chlorination as the only treatment; the conclusions of the turbidity study thus apply only to such systems. Results indicated that disinfection efficiency (log-fold decrease in coliform numbers) was negatively correlated with turbidity and was influenced by season, chlorine demand of the samples, and the initial coliform level. Total organic carbon was found to be associated with turbidity and was shown to interfere with maintenance of a free chlorine residual by creating a chlorine demand. Interference with coliform detection in turbid waters could be demonstrated by the recovery of typical coliforms from apparently negative filters. The incidence of coliform masking in the membrane filter technique was found to increase as the turbidity of the chlorinated samples increased. The magnitude of coliform masking in the membrane filter technique increased from <1 coliform/100 ml in water samples of <5 NTU to >1 coliform/100 ml in water samples of >5 NTU. Statistical models were developed to predict the impact of turbidity on drinking water quality. The results justify maximum contaminant levels for turbidity in water entering a distribution system as stated in the National Primary Drinking Water Regulations of the Safe Drinking Water Act.

A procedure has been developed to detect false-negative reactions (interference) in the standard most probable number (S-MPN) technique for coliform enumeration of potable water supplies. Coliform interference was found to occur in the presumptive, confirmed, and completed tests of the S-MPN technique. When coliforms were present, interference with their detection occurred in more than 80% of the samples. The inferior nature of the S-MPN was revealed by the 100% increase in the incidence of completed coliform positive drinking water samples obtained with the modified MPN (M-MPN) technique. The M-MPN procedure was also superior to the standard membrane filter technique. Eight different species of coliforms were recovered from false-negative tests including Citrobacter, Enterobacter, Klebsiella, and Escherichia coli in decreasing order of occurrence. The use of the standard MPN technique for monitoring potable water supplies may lead to a false security that the drinking water supply is potable, i.e., free from indicator bacteria.

Since the S-MPN and membrane filtration (MF) techniques can fail to detect some or all of the coliforms in drinking water, a new alternative MPN test was devised that has greatly increased detection frequencies

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and requires only a modest increase in efforts over the S-MPN. The abbreviated MPN (A-MPN) exhibited a geometric mean coliform recovery 3.5 times greater than either the S-MPN or MF techniques. The A-MPN uses lauryl tryptose broth (LTB) as the presumptive and secondary completion broth media. Gas positive as well as turbid but gas negative LTB tubes are streaked directly onto m-endo agar LES. Brilliant green lactose bile broth is not used.

This report was submitted in fulfillment of Cooperative Agreement No. 906287 by Oregon State University under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period October 10, 1978 to April 9, 1981, and work was completed as of April 9, 1981.

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Most of the experimental efforts were conducted by the following individuals: Dr. Thomas M. Evans, Mr. Mark W. LeChevallier, and Mr. C. E. Waarvick. The invaluable discussions, suggestions, and guidance from the project officer, Dr. Harry D. Nash are gratefully acknowledged.

SECTION 1

INTRODUCTION

The National Interim Primary Drinking Water Regulations promulgated on December 24, 1975 in accordance with the Safe Drinking Water Act (P.L. 93-523) established a maximum contaminant level (MCL) for turbidity in drinking water (1). A turbidity of 1 nephelometric turbidity unit (NTU) was recommended and up to 5 nephelometric turbidity units were allowed if the supplier could demonstrate that turbidity did not interfere with disinfection, prevent the maintenance of effective disinfectant residual in the distribution system, or interfere with microbiological determinations. The regulations also required that daily turbidity samples be taken from representative entry point(s) to the water distribution system, that measurements be made with a nephelometer, and if a turbidity over 1 NTU is obtained, that the measurement be confirmed by resampling the water, preferably within 1 hour.

Turbidity in water is caused by the presence of suspended matter such as clay, silt, organic and inorganic matter, plankton and other microscopic organisms (2,3). Turbidity is an expression of the optical property of water that causes light to be scattered; it is measured by determining the degree of light scattering by particulates present in the samples.

Previous research concerning the effects of turbidity on drinking water potability have associated coliforms with nematodes, crustaceans, iron rust, and plankton inside the distribution system (4,5,6,7). Water that was experimentally contaminated with feces containing infectious hepatitis and then chlorinated, produced illness in volunteers, while a similar water sample that was coagulated and filtered to remove turbidity and then chlorinated produced no illness (8). Unpleasant odors and tastes have been associated with high turbidities (9). Turbidity can carry nutrients to support microbial regrowth in the distribution system (10,11); the ensuing high standard plate counts can mask the detection of coliforms (10,11,12,13,14,15). Despite these observations, no experimental evidence exists as to the impact of turbidity at entry points to a distribution system practicing no treatment other than chlorination. Since many small communities do not practice full water treatment, one of the purposes of this research was to evaluate the quantitative impact that turbidity has on disinfection efficiency, maintenance of a chlorine residual, and microbiological determinations and to relate these findings to the 1 and 5 NTU maximum contaminant levels.

Enumeration of the total coliform bacterial population by the fermentation tube procedure has been utilized by microbiologists for some 60 years as an indicator of water quality (16). This technique is still utilized for monitoring the quality of potable drinking water supplies throughout the world. In this country, the multiple tube procedure is one of two techniques permitted for monitoring drinking water supplies under the regulations of the Safe Drinking Water Act (1).

Several studies have suggested that the recovery of coliforms by the fermentation tube technique is potentially subject to various kinds of interferences, especially at the presumptive stage (11,12). In potable drinking water supplies, pathogens have been isolated with few if any detectable coliforms present (14,17,18). Geldreich et al. presented results from a community water supply survey involving over 2,400 samples (10). The data revealed a decrease in the percentage of coliform-contaminated samples when the standard plate count (SPC) bacteria exceeded 500 cells/ml. The authors suggested that the SPC bacteria were causing an interference with the detection of gas production by the coliform bacteria. This conclusion is consistent with the earlier studies of Chambers, who demonstrated that a slight reduction in coliform numbers could result in the failure to detect gas production (19).

Interference by bacteria that are antagonistic to coliforms (12,14,15) as well as the inhibitory nature of the media used in the fermentation tube technique have been suggested as factors contributing to the masking of coliform detection (19,20).

In one section of this report a modified MPN (M-MPN) procedure is presented that has allowed the first quantitative assessment of the magnitude of coliform interference in raw surface water supplies and in potable drinking water. Specific steps and media used in the M-MPN were analyzed on a tube basis to develop a logic for an efficient alternative to improve coliform detection.

SECTION 2

CONCLUSIONS AND RECOMMENDATIONS

This study has demonstrated that turbidity in the surface source water is detrimental to drinking water quality in systems which practice no treatment other than chlorine disinfection. Models were developed by multiple linear regression analysis to predict the impact of a specific turbidity level on chlorine demand and the persistence of coliforms. The effect of turbidity on coliform persistance and chlorine demand was noted at all turbidities above 1 NTU. Turbidities over 5 NTU are associated with a chlorine demand in 1 hr of 10 ppm, coliform survival is enhanced, and interference with coliform detection by membrane filtration is readily demonstrable. Elevated turbidities can impede the maintenance of a free residual chlorine in the distribution system and false negative reactions in the MF technique can lead to a false security that the water is potable.

A modified MPN procedure was developed which served as an experimental tool for quantitative evaluation of the standard MPN procedure. The results revealed that the standard MPN detected coliforms in only 50% or less of the contaminated drinking water samples. An abbreviated MPN technique was developed to overcome most of the problems associated with the standard MPN. Field trials revealed that the abbreviated MPN provided significantly increased levels of coliform detection compared to the standard MPN.

Procedures are provided for evaluating the impact of turbidity on chlorine demand, coliform survival, and coliform masking in the membrane filtration technique for surface waters entering a distribution system which does not practice filtration. The results of this study justify maximum contaminant levels for turbidity as stated in the National Primary Drinking Water Regulations of the Safe Drinking Water Act.

Additional field studies are recommended to assess the suitability of the standard and abbreviated coliform detection techniques in other regions of the country. Future efforts are also needed to develop new techniques to increase the sensitivity of coliform detection in drinking water supplies.

SECTION 3

MATERIALS AND METHODS

Sampling area. Most samples were collected from the finished drinking water supply of an Oregon coastal community serving 14,000 residents and from the two protected coast range streams supplying the raw water to the city.

The intake points of the raw water supplies are located behind small concrete retention dams about 1.2 to 1.5 m (4 to 5 ft) high and constructed across the streams. At one intake, water flows by gravity into a 30.5 cm (12 in) main line, and after about 45 min flow time, receives gaseous chlorine injection by a flow proportional device resulting in about 1.5 mg/l total initial chlorine concentration. At the other intake, water is pumped from the diversion dam into a "settling reservoir". The reservoir water effluent receives a similar dose of gaseous chlorine prior to entering the distribution system. The chlorine contact time is approximately 30 min prior to reaching the first service connection. There is no physical or chemical treatment of the raw water except for chlorination.

In the turbidity study, samples were also collected from other watersheds: Oak Creek, and Rock Creek, both of which, like the coast streams, are waters receiving no industrial or domestic wastes. Mary's River and Willamette River receive domestic effluent and agricultural runoff. Due to the extensive winter and spring precipitation periods in the study areas, and other physical properties of the watersheds, rains occasionally carried particulate material into the streams leaving the intake and finished waters turbid. In the development of alternative coliform detection techniques, some 25 samples were also collected from the drinking water distribution system of several western Oregon communities which use ground water and do not practice chlorination.

Collection of samples. Raw water was collected in the area of the municipal intake in sterile polypropylene carboys. During or following periods of storm water runoff the surface water was usually turbid. During the summer, sediment was collected from the stream bottom (top 5-10mm) and added to the surface water to achieve various turbidity levels. There were no differences in analytical relationships (turbidity, chlorine demand, total organic carbon) between samples collected during the summer and supplemented with sediment or samples collected during periods of precipitation.

The chlorine demand of the samples was determined by amperometric titration (2). Chlorine was added to 400-ml portions of the sample so that the final concentrations of added chlorine ranged from 0.05 to 2.5 mg/l. A stock solution of chlorine was prepared daily from calcium hypochlorite (Fisher Scientific Company) and standardized by amperometric titration (Fischer and Porter titrator). The pH values of the samples after chlorine addition were all within 0.2 units of neutrality. Samples were incubated in the dark for 1 h at 10°C. The incubation temperature was comparable to the mean yearly temperature of the surface water supplies. At the end of this time interval, free and total chlorine residuals were determined using a Fischer and Porter amperometric titrator. Samples retained for microbiological analysis were dechlorinated using sodium thiosulfate (Mallinckrodt). The difference between the amount of chlorine added and the amount remaining after 1 h contact is termed the chlorine demand of the water. The turbidity of the samples was measured using a Hach model 2100A turbidimeter. Formazin turbidity standards were prepared weekly according to Standard Methods (2). Chlorinated, turbid water samples were blended for 1 minute in 15 second intervals at 20,000 rpm in a Waring 700 Commercial Blendor. Zwittergent 3-12 (Calbiochem) was added to water samples before blending (10^{-6} M final concentration) to help disperse bacteria. Nitrate, orthophosphate, suspended soilds, and total organic carbon content of the water were all determined according to Standard Methods (2).

Finished drinking water samples were collected in 4-liter sterile polypropylene containers. Sodium thiosulfate was added (2) to neutralize any chlorine residual in the drinking water samples. Free and combined chlorine residuals were determined with a Hach field test kit Model CN-70 using the N,N-diethyl-p-phenylenediamine (DPD) colorimetric techniques (2). Temperature of all water samples was determined upon collection with a YSI-Telethermometer 400 series. Samples were placed on ice and transported back to the laboratory within 3 h after collection and analyses were completed within 7 h.

Microbiological techniques. Unless otherwise noted, all bacteriological techniques, and time and temperature of incubation conformed to Standard Methods (2) and to the procedures of the Microbiological Methods for Monitoring the Environment (21). In some portions of the study a 9-tube MPN was used. Standard plate count bacteria were enumerated using the MF technique (22).

In the standard multiple tube fermentation technique, volumes of 10, 1.0, and 0.1 ml were inoculated into two sets of 9 or 15 tubes. One set contained Lactose Broth (LB; Difco lot Nos. 652242 and 662331) and the other set Lauryl Tryptose Broth (LTB; Difco lot No. 662637). Both media were supplemented with phenol red (Sigma) indicator 18 mg/l. The confirming medium was Brilliant Green Lactose Bile Broth (BGLB; Difco lot No. 666632). The completed test agars were Eosin Methylene Blue (EMB; Difco lot No. 610498) and m-Endo agar LES (M-LES; Difco lot No. 663068). Typical and atypical colonies were picked from both EMB and M-LES agar media and streaked onto slants of Tryptic Soy Yeast Extract

Agar (TSYA), containing Tryptic Soy Broth (Difco lot No. 656810) and 0.3% yeast extract (Difco lot No. 668517). After a 24 h incubation at 35°C, growth from the slant was removed for Gram staining and transferred into secondary broth tubes of LB and LTB.

In the M-MPN procedure the same media were employed as in the standard most probable number (SMPN) technique, except EC broth (Difco lot No. 641057) was added as an additional confirmatory broth and incubated at 35°C. The M-MPN scheme consisted of the S-MPN technique plus additional manipulations designed to recovery coliforms from any tests which were negative (Fig. 1). In the M-MPN scheme, coliform masking or interference was defined as the recovery of a completed coliform in pure culture from a tube or plate which failed to yield a coliform by the S-MPN procedures. Masking could have occurred at any of the three stages in the S-MPN scheme. An increase in the coliform MPN index would occur whenever a completed coliform was obtained from the M-MPN and not from the corresponding S-MPN scheme.

In the M-MPN procedure, acid and/or turbid (gas-negative) presumptive tubes were subcultured onto m-LES and EMB and 1 ml was transferred to sterile tubes of BGLB and EC broths (Fig. 1). If gas was produced in either BGLB or EC, the Standard Method's completed test was performed using both m-LES and EMB agar media. When either presumptive or confirmatory broths were subcultured onto m-LES or EMB plates, two procedures were used to determine whether growth on the plates contained coliforms. One procedure, designated isolated colony (IC), conformed to the Standard Method's procedure and consisted of transferring one isolated colony from the plate to secondary tubes of LB and LTB broth. If gram-negative gas-producing (LB or LTB) bacteria were recovered, masking in the presumptive tube was demonstrated. In the second procedure, designated multiple inoculation (MI), growth from the heavy portion of the streak or 2 to 5 isolated typical coliform colonies were transferred into LB and LTB broth tubes. Tubes with gas were again streaked back onto sterile plates of m-LES and processed by the IC procedure (Fig. 1, presumptive test). Isolated, typical coliform colonies were picked and inoculated into LB and LTB and TSYA for Gram staining to fulfill the concept of Koch's postulates, i.e., gas formation from lactose by a pure culture of a Gram-negative rod. The MI procedure was the most efficient way to detect coliforms from false-negative presumptive tubes or to detect coliforms which did not produce gas in BGLB.

Masking could also be demonstrated in the confirmed (BGLB) medium. Positive presumptive tubes were subcultured into BGLB and EC media. If the BGLB tube was negative, the positive presumptive tube was processed as described previously through Step I of the M-MPN scheme (Fig. 1). Coliform masking was positive if the S-MPN confirmatory step was negative and completed coliforms were recovered by either Step I of the M-MPN scheme and/or from the EC confirmatory broth.

Masking in the completed step was demonstrable in samples which contained presumptive and confirmed positive tubes but failed to yield

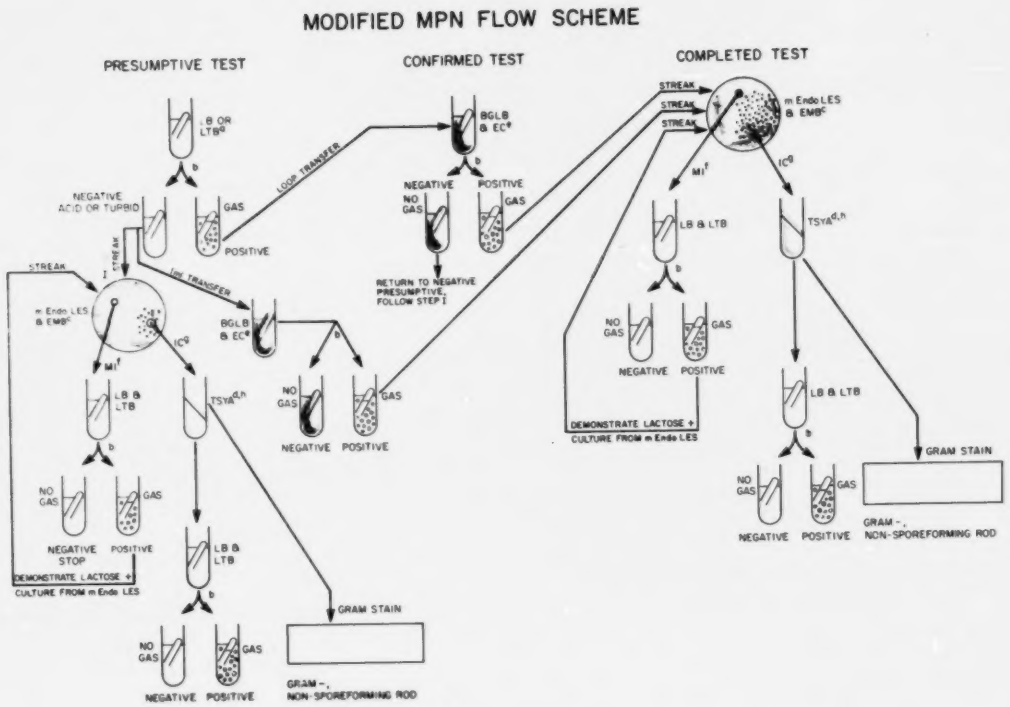

Fig. 1. Flow scheme for modified most probable number procedure. Abbreviations: a, lactose broth (LB) and lauryl tryptose broth (LTB); b, tubes examined after 24 and 48 h at 35°C; c, m-Endo agar LES and eosin methylene blue (EMB) agar; d, incubated for 24 h at 35°C; e, brilliant green lactose bile (BGLB) broth and EC broth; f, multiple inoculation (MI) technique; g, inoculation of an isolated colony (IC); h, tryptic soy yeast extract agar (TSYA). See Material and Methods for further explanations.

gram-negative gas-producing rods when single, isolated "typical" colonies were picked into secondary lactose broth tubes. When typical completed coliform isolates were recovered from the positive presumptive or positive confirmed tubes and not from the completion agar media, masking was demonstrated in the S-MPN completed test stage.

The M-MPN index was calculated on the basis of a compilation of positive completed tests from both the S-MPN and M-MPN techniques. The magnitude of masking in the standard technique was calculated from the difference in the MPN indices (total coliforms/100 ml) derived from the S-MPN and M-MPN tube profiles.

Membrane filter (MF) enumeration of total coliforms was conducted using Gelman GN-6 membranes (pore size 0.45 μm) and m-Endo agar LES (Difco lot No. 663068). Duplicate volumes of 250 and 100 ml of drinking water and 10 and 1 ml volumes of untreated surface water were routinely analyzed. Volumes of 250 ml were usually necessary to get sufficient coliform numbers for identification purposes. All numerical comparisons were based on the results of filtering 100 ml volume of drinking water. Typical colonies were randomly selected for verification. In samples where the number of typical colonies was less than 20 per plate, colonies were picked from replicate plates. In this case, when possible, a total of ten colonies were submitted to the verification scheme (21).

Coliform masking in the MF technique. Interference with coliform detection in the membrane filter technique was demonstrated by placing filters (100 ml volume of sample analyzed) with no typical green sheen colonies into tubes of LTB and incubating up to 48 h at 35°C (Fig. 2). Turbid tubes were subcultured onto m-Endo agar LES. Typical green sheen colonies were inoculated into tubes of LTB and LB by either the multiple inoculum (MI) or isolated colony (IC) method; incubated for 24 to 48 h, and examined for gas production. Pure cultures were maintained on slants of TSYA. Coliforms were identified by methods described below. A most probable number (MPN) index was generated by filtering triplicate volumes of 3-log dilutions and processing the filters in the manner described above. The magnitude of masking was determined by subtracting the original MF verified count from the MPN. We recognize the potential dangers of using these different techniques to measure the efficiency of coliform recovery. However, a side-by-side comparison was essential in order to estimate the differential affect that turbidity has on the MF technique.

Identification of total coliform bacteria. Coliforms from the greatest sample dilutions were selected for identification. Three coliforms were identified from the S-MPN technique where LTB was the presumptive medium and 3 where LB was the presumptive medium. Coliforms isolated from each masked test were also identified.

Coliforms were identified by use of triple sugar iron agar slants (Difco), the IMViC (indole, methyl red, Voges-Proskauer, citrate) tests, lysine and ornithine decarboxylase broths, arginine dihydrolase broth, and mucate and malonate fermentation. Cultures from the American Type

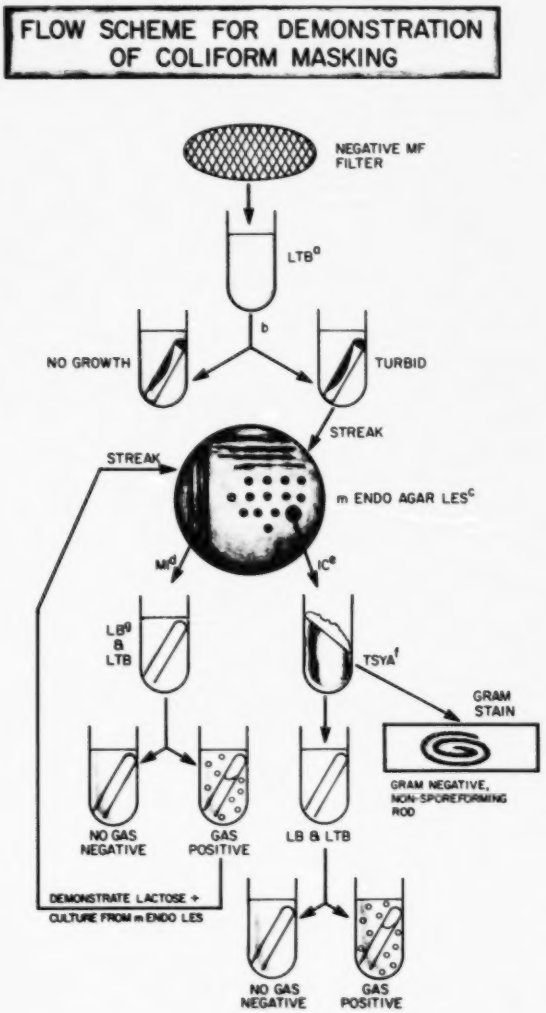

Fig. 2. Flow scheme for demonstrating coliform masking in the MF technique. Abbreviations: a, lauryl tryptose broth (LTB); b, tubes examined after 48 h at 35°C, c, m-Endo agar LES; d, inoculation of multiple colonies from heavy growth portion of the plate (MI); e, inoculation of an isolated colony (IC); f, tryptic soy yeast extract agar (TSYA); g, lactose broth (LB).

Culture Collection were used as a quality control procedure to ensure that proper reactions were obtained in the media and to aid in the identification of the coliform isolates. In addition, the API 20E system (Analytab, Plainview, N.Y.) was used to confirm the identities of 10% of the isolates.

Statistical analyses. Physical, chemical, and microbiological parameters were entered in the Oregon State University CYBER 70/73 computer equipped with a statistical interactive programming system (SIPS) for data storage and statistical analyses. The SIPS program produces a standard multiple regression model with the form $Y = B_o + B_1X_1 + B_2X_2 + \ldots B_nX_n + e$ in which Y is the dependent variable, B_o is the Y intercept, B_{1-n} are regression coefficients, X_{1-n} are independent variables, and e is an error term (17). Some statistical comparisons were also made on the basis of the paired t-test on logarithmically transformed data (23).

Quality assurance. A quality assurance program as outlined in Microbiological Methods for Monitoring the Environment (21) was used throughout the course of this study. The laboratory distilled water supply was monitored for chemical and bacterial quality and suitability for use in culture media. Glassware was checked for detergent and other inhibitory residues. Each culture medium was checked for proper pH, performance and sterility. Temperatures of autoclaves and incubators were monitored on a per use basis. Technician performance in conducting the analyses and in counting of membrane filter total coliform colonies was monitored and controlled. Lot numbers of media used throughout this study were also recorded. The frequency at which the various quality control parameters were tested conformed to that given in reference 21.

SECTION 4

RESULTS

Impact of turbidity on disinfection. The influence of turbidity on drinking water quality was examined in six watersheds where turbidities at entry points to distribution lines ranged from 0.2 to 15.0 NTU (geometric mean of 2.4 NTU). Coliform densities in raw water ranged from 11 to 500 coliforms/100 ml (geometric mean of 88 coliforms/100 ml) and were reduced 1 to 3 logs (geometric mean of 1.8 logs) by chlorination.

The nature of the turbidity in surface water samples was characterized for two of the watersheds studied. The turbidity was found to be mainly inorganic in nature with clay particles comprising the greatest fraction of the inorganic material. The clay minerals were identified as montmorillonite, mica, and vermiculite. Montmorillonite was present in the greatest quantity. The turbidity found in the drinking water distribution system supplied by the two watersheds was similar in nature to that found in the untreated surface water source.

The relationship between turbidity and chlorine disinfection efficiency was determined by measuring the decrease in numbers of coliforms at different turbidity values and different concentrations of added chlorine (Fig. 3). The results indicated that coliforms in high turbidity water (13 NTU) were reduced to only 20% of the initial count while coliforms in low turbidity water (1.5 NTU) were undetectable even when large volumes were sampled (3 logs or greater decrease). Disinfection efficiency (log fold decrease in coliform numbers, LFDC) was found to be negatively correlated with turbidity ($r = 0.777$, $p < 0.01$).

Turbid water samples were examined in an attempt to determine factors which aid bacteria in surviving exposure to chlorine. Scanning electron photomicrographs demonstrated that some bacteria were either embedded in turbidity particles and/or appeared to be coated with amorphous material. Blending of chlorinated turbid water increased the number of standard plate count bacteria as much as 5 times indicating the physical separation of cells attached to common particles. Blending did not increase coliform counts as dramatically and increased counts were not observed with all samples.

The chlorine demand (CLDMD) of the surface water supplies was found to be positively correlated with both the turbidity and total organic carbon (TOC) content of the water (Fig. 4 and 5). The association of CLMD with turbidity and TOC could be described by the equations; CLDMD =

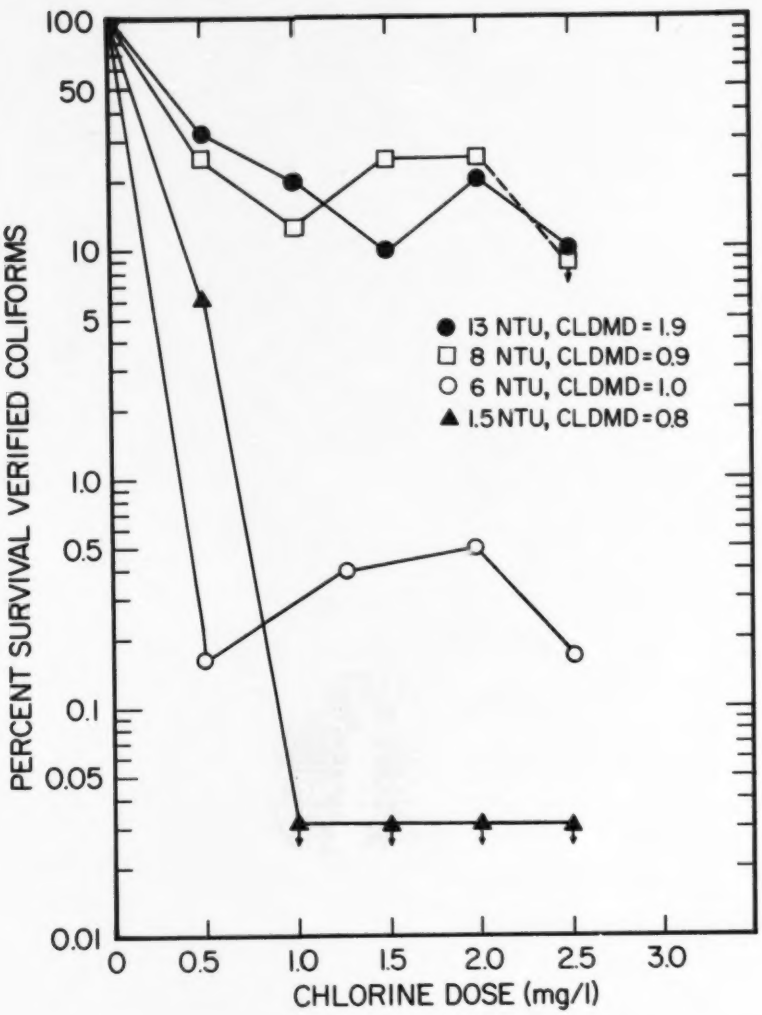

Fig. 3. Coliform persistence in turbid chlorinated water. Abbreviations: NTU, nephelometric turbidity units; CLDMD, chlorine demand. Exposure to chlorine was for 1 hr at 10°C.

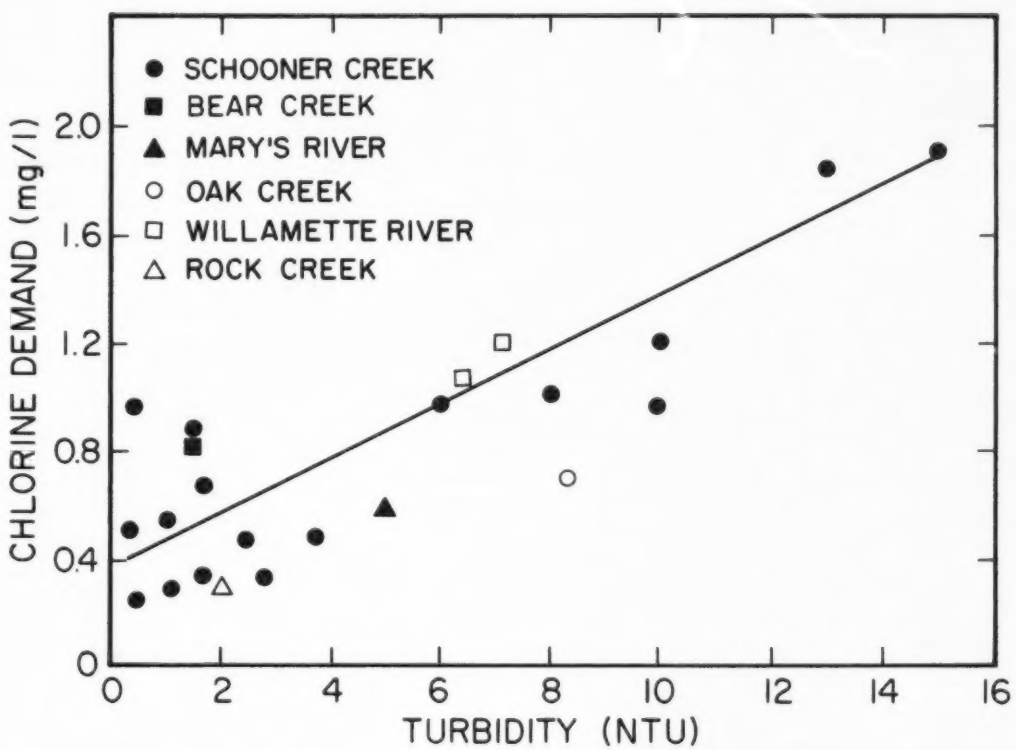

Fig. 4. Relationship between turbidity and chlorine demand in samples collected from 6 watersheds in western Oregon.

0.040 + 0.086 NTU (r, 0.85; p <0.01), and CLDMD = 1.36 + 0.525 (r, 0.95, p <0.01), where TOC is measured in mg/1. Experiments were performed to determine why CLDMD was associated with both TOC and turbidity. Turbid samples were filtered using prewashed filters (Millipore GS; 0.22 µm pore size). Chlorine demand and TOC levels were determined on the sample before filtration and on the filtrate. The filtrate exhibited a 99.9% reduction in turbidity but retained 90% of the chlorine demand of the unfiltered sample. These experiments demonstrated that the chlorine demand of raw water is almost totally associated with soluble TOC. The correlation between turbidity and chlorine demand may be explained by the high degree of association of turbidity and TOC (r = 0.82; p <0.01) (Fig. 6).

The positive correlation of chlorine demand and turbidity indicated that turbidity and its associated TOC could potentially impact the maintenance of a free chlorine residual in the distribution system. Turbidity which entered the distribution system and contained material with an unsatisfied chlorine demand would reduce the chlorine residual in the distribution system.

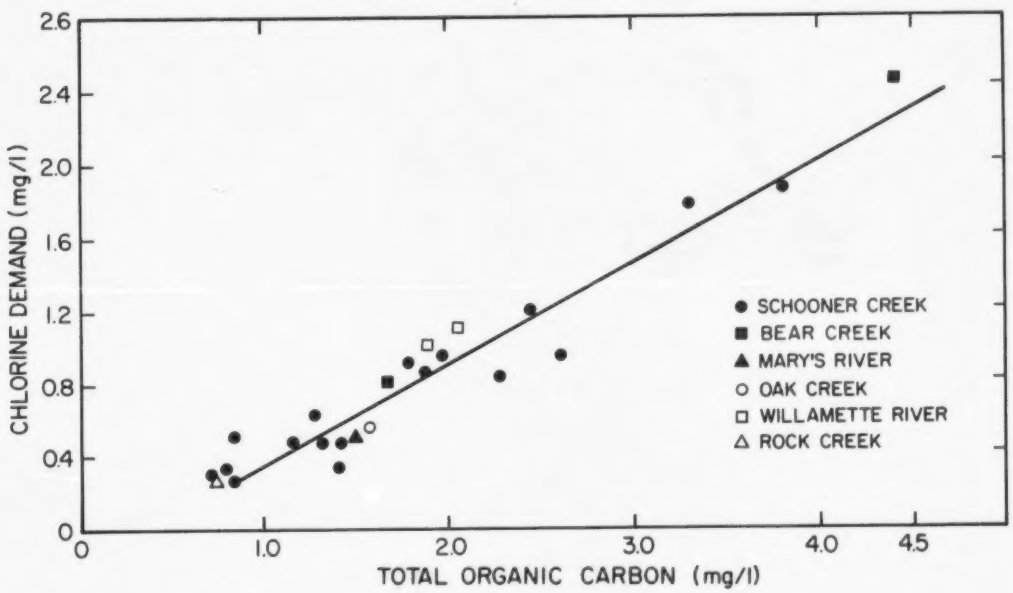

Fig. 5. Relationship between chlorine demand and total organic carbon in turbid water samples collected from 6 watersheds in western Oregon.

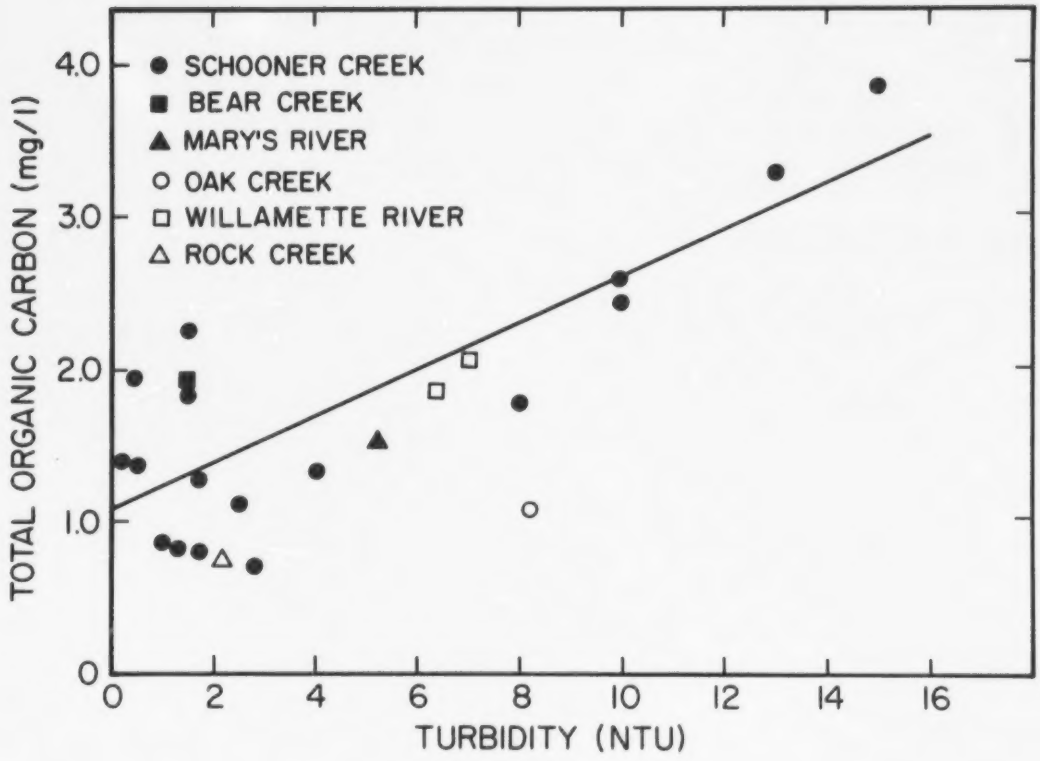

Fig. 6. Relationship between total organic carbon and turbidity in samples collected from 6 watersheds in western Oregon.

Statistical models explaining the impact of turbidity. Statistical models were developed through multiple linear regression computer analysis to define the interrelationships between turbidity, chlorine demand (CLDMD), disinfection efficiency (LFDC) and TOC. The models obtained should allow predictions as to how turbidity impacts drinking water quality. Disinfection efficiency, hence efficacy of the treatment process, was found to be influenced by season, turbidity, chlorine demand, and the initial coliform density in the raw souce water (Table 1). The coefficient of multiple determination R^2, expressed as a percent, for Model 3 (Table 1) indicated that 66% of the variation in disinfection efficiency was explained by the variables in the model. The inclusion of a numerical term which describes the impact of season on TOC concentration was necessary because of the rainfall patterns in the Pacific Northwest region. The TOC concentration in the streams would gradually increase in the summer months of low rainfall amounts due to the presence of

vegetation debris in the watersheds. The TOC concentrations continued to increase in November and December when stormwater runoff carried accumulated organic debris into the receiving stream. In January and February, and after these initial rainfall events, the amount of succeeding stormwater run off was sufficient to increase the stream flow rate and result in reduced TOC concentrations. Unaccounted for variation in Model 3 may be due to unmeasured parameters such as seasonal variations in coliform populations, varying sensitivities of coliforms to chlorine, and coliform masking in turbid samples. The statistical significance of the model may be evaluated on the basis of R^2, mean square error (MSE), total squared error (C), standard error of the regression coefficient, and the Student's t-values. Based on these criteria, the model was significant ($p < 0.01$) and unbiased (24).

Chlorine demand which was important in predicting disinfection efficiency was found to be a function of the turbidity and the associated TOC content of the water sample (Table 1). The variables turbidity and TOC explained 94% (R^2, Table 1) of the variation in CLDMD. Turbidity was found to be an accurate predictor of TOC levels in the raw water ($r = 0.83$, $p < 0.01$). These models were based on results obtained from six watersheds. In developing the three models, the data obtained from each of the watersheds were not found to be significantly different.

Impact of turbidity on microbiological determinations by membrane filtration. Turbidity was also found to be associated with failures to detect coliforms by the MF technique. The MF coliform failures were assessed by placing filters (100 ml volume of sample analyzed) without typical colonies (often without any visible colonies) into tubes containing LTB and processing in a manner similar to the M-MPN method. The incidence of false-negative results or coliform masking in the MF technique increased as turbidity increased (Fig. 7). At low turbidities (1 NTU-2 NTU), 16% of the filters sampled which were initially free of visible typical colonies were found to be coliform positive. In the NTU range of 0-2, six samples contained less than 0.6 NTU and of these, one sample exhibited masking. The remaining 30 samples were over 1 NTU and 4 were masked. The frequency of false negative results increased to 45% at 5 NTU and to over 80% at turbidities in excess of 10 NTU. The magnitude of false negative results in the MF technique was determined by filtering triplicate sample volumes of 3-log dilutions and processing by the M-MPN technique. A comparison of the number of coliforms recovered on the membrane filters by conventional techniques to the value of the M-MPN index developed from the filters placed into LTB allowed an estimation of the magnitude of masking. The magnitude of MF coliform masking as determined with the aid of the MPN-procedure is only an estimate due to the lack of precision in the MPN technique and can be used only to demonstrate the potential for coliform masking. The average magnitude of masking (coliform underestimation) was less than 1 coliform/100 ml for 83% (10/12) of the samples having turbidities below 5 NTU, while the magnitude of masking was greater than 1 coliform/100 ml for 75% (6/8) of the samples having turbidities higher than 5 NTU. MF coliform counts were underestimated by as much as 2- to 20-fold in drinking water samples with turbidities in the range of 5 to 10 NTU. Citrobacter freundii

Table 1. Models derived to predict impact of turbidity on drinking water quality.

Model	Coefficient of Multiple Determination (R^2)	Number of Observations (N)	Mean Squared Error (MSE)	Total Squared Error (C)	Standard Error of Regression Coefficients	t Values
1) TOC[a] = 1.070 +0.153 (NTU)[b]	-[c]	23	-	-	-	-
2) CLDMD[d] = -0.075	0.936	17	0.0186	3.0	0.0989	-0.77
+0.029 (NTU)					0.0413	2.02
+0.405 (TOC)					0.0780	5.09
3) LFDC[e] = 1.6951	0.663	32	0.1965	4.5	0.2448	6.92
+0.0549 (Season)[f]					0.0257	2.13
-0.1676 (NTU)					0.0325	-5.15
+0.7763 (CLDMD)					0.3829	2.03
+0.0003 (TC)[g]					0.0011	2.76

[a] TOC, Total organic carbon in mg/l.
[b] NTU, Nephelometric turbidity units.
[c] Multiple regression terms do not apply to linear regression involving one independent variable (24).
[d] CLDMD, Chlorine demand in mg/l.
[e] LFDC, Log-fold decrease in coliforms.
[f] A numberical term [1-12 for January (1) to December (12)] used to explain seasonal effects on LFDC.
[g] TC, verified total coliforms measured by MF technique.

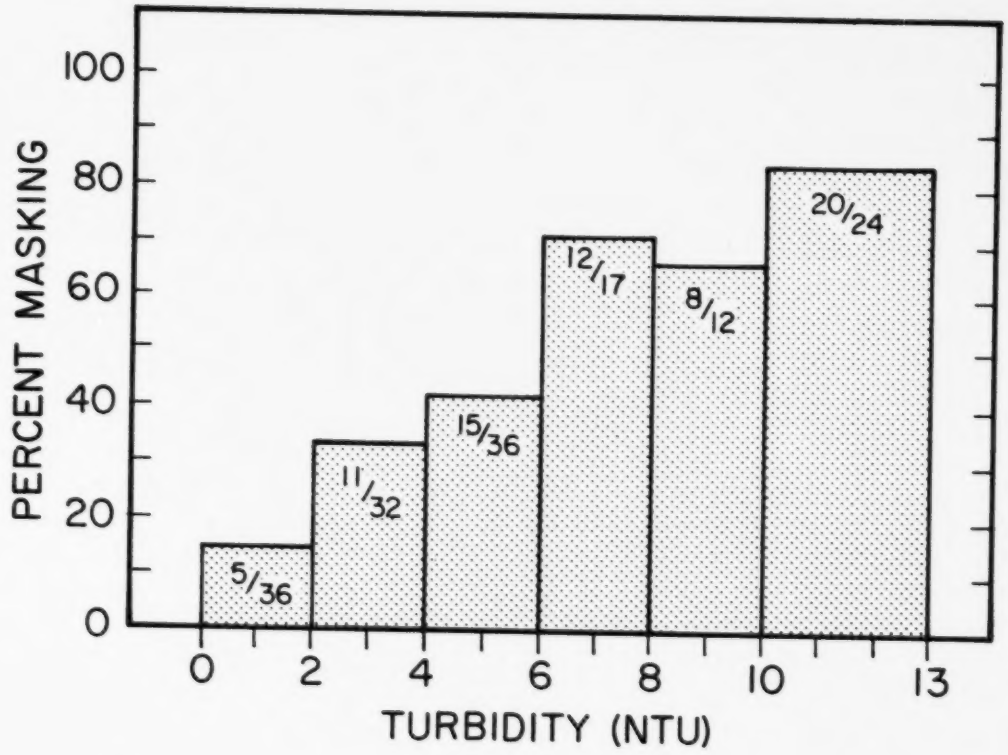

Figure 7. Relationship between percent of filters with masked coliforms and turbidity in chlorinated water (1.0 to 1.6 mg/l free chlorine). The number of filters with masked coliforms are presented over the total number of filters analyzed.

comprised 60% (32/54 isolates) of the coliforms isolated from masked samples. Enterobacter spp (E. cloacae, E. agglomerans and E. aerogenes) were the second most commonly isolated group comprising 30% (16/54 isolates) of the coliforms isolated from masked samples.

Inadequacies of the S-MPN technique. Interferences with detection of coliforms occurred at all tests in the S-MPN technique (Table 2). When analyzed on a sample to sample basis, most of the interference occurred at the presumptive test whether LB or LTB was used as the presumptive medium. The second most common incidence of interference was noted with the confirmatory medium, BGLB. The problems with presumptive and confirmatory media were recognized when completed coliforms were obtained from gas negative tubes of LB, LTB, and BGLB.

The incidence of masking in the various tests recorded on a sample basis did not reveal the numerical affects determined by the geometric mean coliform recovery. For example, in drinking water, when coliforms were detected, the geometric mean of the M-MPN revealed 4.6-fold more coliforms than the S-MPN and 5-fold more coliforms than the MF technique ($p < 0.005$). There was no significant difference in geometric mean coliform recovery by LTB or LB ($p < 0.5$) in the S-MPN or in the M-MPN technique (Table 3).

The magnitude of coliform masking (the difference between the M-MPN and S-MPN coliform indices), was not found to be correlated with either the temperature of the water samples or precipitation events in the water-shed when the samples were collected from the distribution system. The lack of association of coliform masking in the S-MPN technique with drinking water turbidity was distinct from the correlation found with the MF technique.

The most commonly recovered coliform species from false-negative presumptive and confirmed tests of drinking water were C. freundii and Enterobacter species which comprised about 80% of the total isolates identified. Escherichia coli, K. pneumoniae, E. aerogenes, E. agglomerans, E. cloacae, H. alvei and C. diversus each comprised approximately 3% of the isolates also recovered from false-negative S-MPN tests.

Comparison of the S-MPN, M-MPN and MF techniques for the enumeration of coliforms. The underestimation of coliforms by the S-MPN technique in drinking water indicated that in 92% (34/37) of the occasions when coliforms were detected, the M-MPN index was greater than the S-MPN index (Fig. 8). In addition, 30/37 of the M-MPN indices were greater than the upper limit of the 95% confidence interval of the S-MPN values.

The advantage of the M-MPN technique relative to the S-MPN and MF techniques in detecting coliforms in drinking water samples which appeared coliform-free, is documented in Tables 4 and 5 and Fig. 9. The M-MPN technique increased the incidence of coliform positive drinking water samples over the S-MPN by 100% regardless of the presumptive broth used (Table 4). The MF technique recovered verified coliforms in 23 drinking

Table 2. Incidence of masking in the presumptive, confirmed, or completed tests in the standard most probable number technique based on analysis of 100 drinking water samples.

S-MPN step affected	Presumptive media used in parallel analysis			
	Lactose Broth		Lauryl tryptose broth	
	Samples unmasked at each test	Percent unmasked	Samples unmasked at each test	Percent unmasked
Presumptive	29	85[a]	18	58[b]
Confirmed	14	41	18	58
Completed	4	12	4	13

[a] A total of 34 samples exhibited masking when LB was the presumptive medium.

[b] A " " 31 " " " " " LTB " " " "

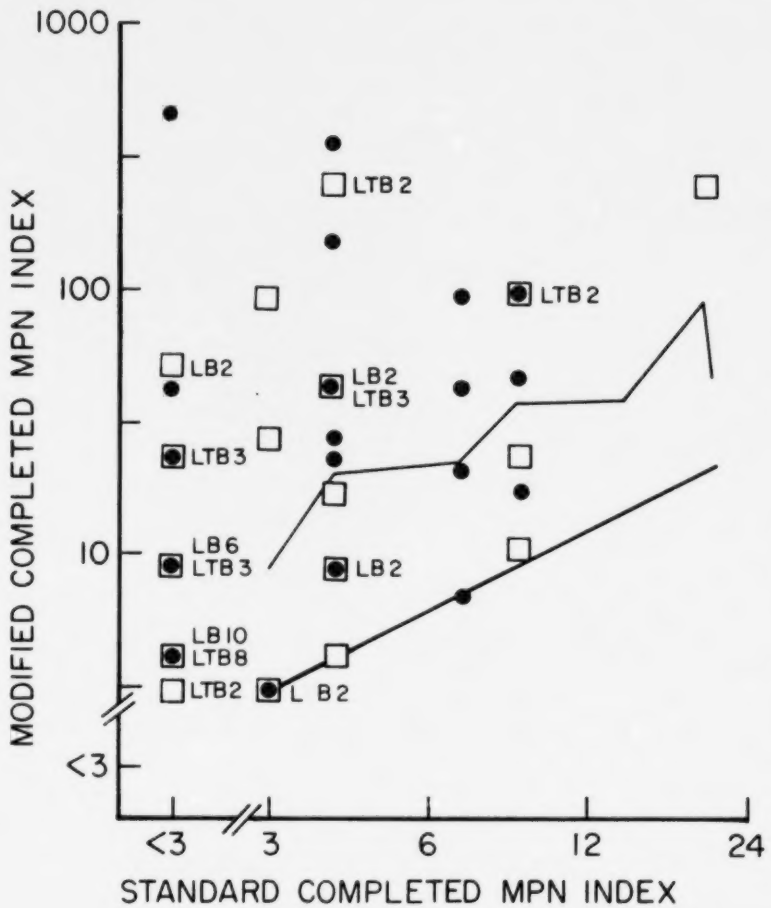

Fig. 8. Relationship between the standard and modified most probable number MPN techniques when used to detect coliforms in potable drinking water samples. Most probable number analyses conducted in parallel using lactose broth (LB; ○) and lauryl tryptose broth LTB;□) as the presumptive media. Numbers following data points indicate number of occurrences. The straight line represents the line of theoretical equility and the jagged line the upper 95% confidence limit of the S-MPN value.

Table 3. Geometric mean number of total coliforms enumerated by various techniques from drinking water samples[a].

Geometric mean total coliforms per 100 ml				
S-MPN[b]		M-MPN[c]		
Presumptive media used in parallel analysis		Presumptive media used in parallel analysis		
Lactose broth	Lauryl tryptose broth	Lactose broth	Lauryl tryptose broth	Membrane filter
2.1	2.2	9.0	11.0	2.0

[a]Means calculated only for samples when coliforms were detected by any technique.
[b]S-MPN, completed standard most probable number.
[c]M-MPN, completed modified most probable number.

Table 4. Comparison of the standard and modified most probable number techniques for the detection of coliforms in 100 drinking water samples.

Presumptive media used in parallel analysis	Comparison	No. of Samples
LTB	S-MPN[a] coliform (+)	16
	M-MPN[b] coliform (+)	33
	M-MPN index greater than S-MPN index	31
	M-MPN coliform (+), S-MPN coliform (-)	17
LB	S-MPN coliform (+)	17
	M-MPN coliform (+)	37
	M-MPN index greater than S-MPN index	34
	M-MPN coliform (+), S-MPN coliform (-)	20
LTB or LB	M-MPN coliform (+), S-MPN coliform (-)	19
	M-MPN index greater than S-MPN index	38

[a]S-MPN, standard most probable number technique completed through eosin methylene blue and/or m-Endo LES agar media followed by LTB and/or LB.
[b]M-MPN, modified most probable number technique completed as in note a.

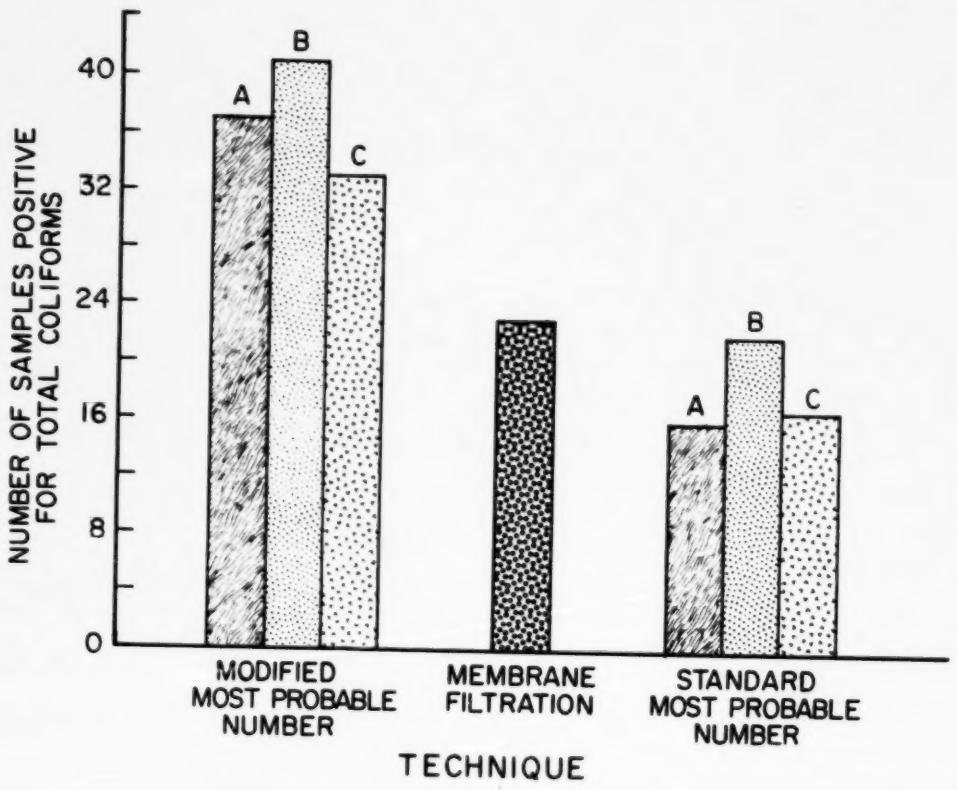

Fig. 9. Number of potable drinking water samples in which coliforms were recovered by the membrane filter, standard most probable number (MPN), and modified MPN techniques. The MPN analyses are expressed as the results obtained when lauryl tryptose broth (A) or lactose broth (C) were used in parallel analyses and as the total number of samples (B) in which coliforms could be recovered by either presumptive medium.

Table 5. Comparison of the standard membrane filter to the standard most probable number and modified most probable number techniques for the detection of coliforms in 100 drinking water supplies.

Presumptive media used in parallel analysis		No. of Samples
LTB	S-MPN[a] coliform (-), MF[c] coliform (+)	11
	S-MPN[b] coliform (+), MF coliform (-)	3
	M-MPN[b] coliform (-), MF coliform (+)	2
	M-MPN coliform (+), MF coliform (-)	12
LB	S-MPN coliform (-), MF coliform (+)	10
	S-MPN coliform (+), MF coliform (-)	6
	M-MPN coliform (-), MF coliform (+)	1
	M-MPN coliform (+), MF coliform (-)	15
LTB or LB	M-MPN coliform (-), MF coliform (+)	0

Footnotes a and b explained in Table 4.

[c]MF, membrane filter technique.

water samples, the S-MPN from 22 samples, and the M-MPN detected completed coliforms in 41 samples (Fig. 9). The M-MPN, when both presumptive media were used, never failed to detect coliforms when MF verified coliforms were detected (Table 5). However, there were 18 samples which contained completed coliforms by the M-MPN technique and no MF verified coliforms were detected. The latter results are surprising since MF sample size was 100 ml while the M-MPN utilized 33.3 ml.

Coliform masking in the S-MPN technique was found to affect compliance with the Safe Drinking Water Act Regulations (Table 6). During the 8 months in which one community's drinking water supply was monitored, the results obtained by employing the M-MPN technique indicated that for 6 months coliforms were present in excess of the allowable limit (1). However, both standard techniques (MPN and MF) indicated non-compliance for only 3 months. Non-compliance in one month (2/79) was the result of coliforms being detected by the MF technique in a single sample. The M-MPN technique detected coliforms in this sample (2/79) in all 3 LB presumptive tubes inoculated. If 5 tubes/dilution had been used, the M-MPN technique would probably have indicated non-compliance for this month as well.

Coliforms from drinking water were recovered by all methods most frequently in samples taken at dead-end distribution line sites. The results of coliform analyses of drinking water (Fig. 8, Tables 3,4, and 5) do not represent a random sampling of the water supply. It is recognized

that the physical, chemical, and bacteriological conditions in dead-end lines do not represent all conditions within the distribution system. However, since coliforms were also detected in drinking water samples with free residual chlorine (main line samples), the M-MPN technique is clearly applicable to the isolation of coliforms from all waters within a distribution system and raw waters as well.

Table 6. Impact of using the membrane filter, standard most probable number and modified most probable number techniques on the compliance with the Safe Drinking Water Act regulations.

	Coliform Detection Method				
Date	Membrane filter: coliforms/vol analyzed[a]	S-MPN LB[b]	M-MPN LB[b]	S-MPN LTB[b]	M-MPN LTB[b]
2/79	78/1600[c]	0/48[d]	3/48[d]	2/48[d]	2/48[d]
3/79	3/1600	1/48	1/48	2/48	4/48
4/79	7/1600	1/48	5/48[c]	0/48	2/48
5/79	0/1400	1/42	5/42[c]	4/42	7/42[c]
6/79	9/1900	2/57	14/57[c]	2/57	12/57[c]
7/79[e]	36/2100[c]	7/65[c]	26/65[c]	6/65	19/65[c]
8/79	13/400[c]	3/20[c]	12/20[c]	1/20	15/20[c]
9/79	116/700[c]	5/35[c]	11/35[c]	3/35	13/35[c]

[a]Volumes of 100 ml were sampled and typical colonies submitted for verification. Thus, for 2/79, a total 78 verified total coliforms were obtained from the analysis of 16 samples.

[b]Presumptive media used in parallel analysis.

[c]Indicates occurrences in excess of allowable limit.

[d]Number of positive tubes/total number of tubes inoculated.

[e]Twenty or more samples analyzed this month.

Abbreviated MPN. A confirmation in the limitation of the standard coliform recovery techniques was realized about midway through the current investigation. The M-MPN served as the basis to reveal errors in the standard techniques but was not intended to replace them because of the extensive number of required manipulations. In determining a logical next step to increase coliform recovery, an analysis was made of the M-MPN results. Such analyses revealed that masking in the presumptive stage was common, it confirmed the inhibitory nature of BGLB and confirmed the lack of sensitivity of Levine's EMB in detecting typical coliform colonies at the completed step. As a result of these observations, the

A-MPN was developed and tested based on the following considerations. All turbid but gas negative tubes are to be processed to a confirmatory medium. Due to its availability and good differential capability in revealing presumptive coliform colonies, m-endo agar LES was used as a new confirmatory medium. In order to complete the test, a secondary broth was also used identical in nature to the presumptive medium. The presumptive and secondary medium chosen was LTB. LTB was superior to LB in that fewer turbid only presumptive tubes were obtained with LTB (221 vs 600 for LB in one comparison) and a similar number of masked coliforms were recovered from both LTB and LB.

A somewhat controversial technique was used for comparing coliform recoveries in the A-MPN technique. Recovery of completed, identified coliforms by the isolated colony technique (IC) was compared to the number and type of coliforms initially recovered from the selection of multiple colonies (MI) obtained from m-Endo agar LES, analogous to the procedure used in the M-MPN technique. Consistently greater coliform recoveries were experienced with the MI technique. Further processing of the gas positive MI secondary broth tube by streaking onto m-Endo agar LES always yielded a coliform in pure culture.

In Table 7 a comparison of the geometric mean coliform recoveries from both surface and drinking water samples is presented using various techniques. For surface water the highest geometric mean coliform recovery was obtained with the M-MPN and A-MPN techniques. The geometric mean numbers recovered by the A-MPN technique were intermediate between the standard and modified techniques. The last column of data (CMI, CIC) represents the geometric mean coliform recoveries by the multiple inoculation and isolated colony procedures using two secondary broths (LB and LTB). These data indicated that greatest coliform recovery by the A-MPN technique was obtained with the MI procedure and two secondary broths (LTB and LB). The IC technique which employed both LB and LTB, yielded a coliform recovery comparable to that obtained with the MI procedure and one secondary broth (LTB).

With drinking water, the difference in the geometric mean MPN values are strikingly different by the various procedures. The M-MPN recovered over 5-fold higher geometric mean numbers of coliforms than the S-MPN or MF techniques (Table 7). The A-MPN technique yielded 3.5-fold more coliforms than either the standard MPN or MF techniques. The use of either the CIC or MI procedure in the completed step of the A-MPN technique gave comparable coliform recoveries.

A statistical comparison of mean coliform recoveries from surface waters and drinking waters by various techniques is presented in Tables 8 and 9. In the matrix of t-values, a positive t-value indicates the technique at the top of the Table recovered significantly more coliforms whereas a negative value indicates the technique listed in the left column recovered more. The p-values for the paired t-test analyses indicated the A-MPN technique gave significantly better coliform recovery than both the standard MPN and MF techniques but, was not as successful

Table 7. Number of total coliforms recovered by various detection techniques from untreated surface water and drinking water samples.

	Geometric mean number of total coliforms/100 ml							
			Fermentation tube technique					
					A-MPN			
	Membrane filter verification							
Sample type	LTB	LTB & BGLB	Standard MPN	Modified MPN	LTB MI[c]	LTB IC[c]	LTB and LB CMI[d]	LTB and LB CIC[d]
Surface water[a]	150	88	72	400	220	150	250	230
Drinking water[b]	2.2	1.6	1.6	8.4	5.7	3.4	7.4	5.9

[a] 35 samples examined.

[b] 155 samples examined; 47 were coliform positive.

[c] LTB; secondary medium inoculated by the multiple inoculation (MI) or isolated colony (IC) procedure.

[d] Both LTB and LB secondary media were inoculated by the MI or IC procedure.

Table 8. Statistical analyses of coliform recovery from 35 surface water samples.

	A-MPN MI technique		A-MPN IC technique		S-MPN		M-MPN		MF LTB verification	
	t-value	p-value	t-value	p-value	t-value	p-value	t-value	p-value	t-value	p-value
A-MPN IC technique	3.660	<0.001								
S-MPN	5.486	<0.001	4.275	<0.001						
M-MPN	-4.544	<0.001	-7.557	<0.001	-8.916	<0.001				
MF LTB verification	1.690	0.100	0.138	>0.5	-3.893	<0.001	4.703	<0.001		
MF LTB & BGLB verification	4.683	<0.001	3.158	<0.001	-1.323	0.110	8.444	<0.001	4.926	<0.001

In the matrix of values, a positive t-value indicates the technique at the top recovered significantly more coliforms; a negative value indicates the technique listed in left column recovered more.

Table 9. Statistical analyses of coliform recovery from 47 contaminated drinking water samples.

	A-MPN MI technique		A-MPN IC technique		S-MPN		M-MPN		MF LTB verification	
	t-value	p-value	t-value	p-value	t-value	p-value	t-value	p-value	t-value	p-value
A-MPN IC technique	4.662	<0.001								
S-MPN	9.319	<0.001	6.263	<0.001						
M-MPN	-3.542	<0.001	-6.108	<0.001	-11.208	<0.001				
MF-LTB verification	5.567	<0.001	2.537	<0.001	- 2.131	0.040	8.105	<0.001		
MF LTB & BGLB verification	8.357	<0.001	5.403	<0.001	0.265	>0.5	10.148	<0.001	3.907	<0.001

In the matrix of values, a positive t-value indicates the technique at the top recovered significantly more coliforms; a negative value indicates the technique listed in the left column recovered more.

as the M-MPN technique. In addition, within the A-MPN technique, the MI procedure was superior to the IC procedure for coliform recovery.

The coliform genera recovered from water samples by use of the three fermentation tube techniques is presented in Figure 10. The M-MPN technique detected greater numbers of Citrobacter, Enterobacter, Escherichia and Klebsiella species in samples of untreated surface water than the A-MPN and S-MPN techniques. Relative to the S-MPN technique, the A-MPN technique detected greater numbers of Citrobacter, Enterobacter, and Escherichia and fewer numbers of Klebsiella. The M-MPN and A-MPN also isolated Y. enterocolifican and H. alvei (other category, Fig. 10) more frequently than did the S-MPN technique.

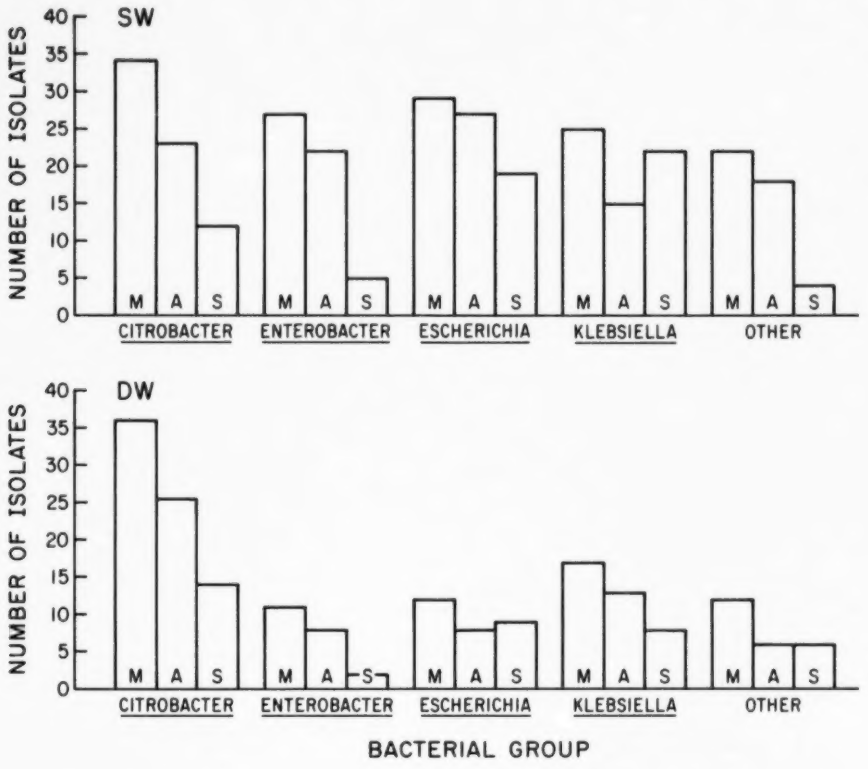

Fig. 10 Frequency of coliform group isolation from untreated surface water (SW) and drinking water (DW) samples by the modified MPN (M), abbreviated MPN (A), and standard MPN (S) techniques.

With drinking water samples, the M-MPN technique detected the greatest number of coliform species in the 5 categories (Fig. 10). The A-MPN technique was superior to the S-MPN technique in detecting Citrobacter, Enterobacter, and Klebsiella species and detected comparable numbers of Escherichia, Y. enterocolitica and H. alvei.

The number of coliform positive drinking water samples as determined by the fermentation tube and MF techniques is presented in Table 10. The M-MPN technique indicated 47 of the 155 drinking water samples examined were coliform positive. The A-MPN technique with the MI completion procedure detected coliforms in 89% (42) of these samples. The use of the IC procedure in the A-MPN reduced the number of apparent coliform positive samples to 32. The standard MPN and MF techniques detected the presence of coliforms in less than 50% of the samples which were coliform positive by the M-MPN technique.

The success and failure of the A-MPN technique in detecting coliform positive drinking water samples is shown in Table 11. The A-MPN technique with the MI completion procedure was more successful and failed fewer times in detecting coliform positive samples than both the standard MPN and MF techniques. The A-MPN procedure failed to detect coliforms in only 5 of the 47 samples which were coliform positive by the M-MPN technique.

The impact of using the standard and newly developed coliform detection techniques to monitor a community's drinking water supply and determine compliance with the Safe Drinking Act regulations is presented in Table 12. With Community A the use of the M-MPN technique indicated non-compliance for 6 of the 13 study months. The S-MPN technique failed to indicate a lack of compliance for the entire study period. The MF and A-MPN techniques were more successful in detecting non-compliance than the S-MPN technique. The A-MPN technique more closely paralleled the results obtained with the M-MPN technique than any of the other coliform detection techniques.

In Community B whose drinking water supply was untreated ground water, only the A-MPN and M-MPN techniques indicated noncompliance with the Safe Drinking Water Act regulations. The number of coliforms detected by the standard MF and MPN techniques for the three study months were within the allowable limits.

Table 10. Number of coliform positive drinking water samples as determined with the abbreviated most-probable-number (A-MPN) standard MPN (S-MPN), modified MPN (M-MPN) and membrane filter (MF) techniques[a].

Detection method	No. Coliform Positive samples
A-MPN; MI technique	42
A-MPN; IC technique	32
S-MPN	10
M-MPN	47
MF; LTB verification	30
MF; LTB and BGLB verification	20

[a]Total number of detectable coliform positive samples was 47.

Table 11. Success and failure of the A-MPN technique in detecting coliforms in drinking water samples compared to other detection techniques.

Detection technique used in parallel	No. samples coliform positive by A-MPN and coliform negative by parallel technique		No. of samples coliform negative by A-MPN and coliform positive by parallel technique	
	A-MPN MI technique	A-MPN IC technique	A-MPN MI technique	A-MPN IC technique
Standard MPN	33	23	1	0
Modified MPN	0	0	5	14
MF; LTB verification	16	12	4	7
MF; LTB and BGLB verification	23	17	1	5

Table 12. Impact of using the S-MPN, A-MPN, M-MPN, and MF techniques on compliance of two communities with the Safe Drinking Water Act regulations[a].

		Coliforms recovered by detection method			
Date	Membrane filter	S-MPN	A-MPN IC technique	A-MPN MI technique	M-MPN
Community A					
8/79	3/400[b]	0/20[c]	2/20[c]	8/20[c,d]	16/20[c,d]
9/79	26/700[d]	1/35	4/35[d]	11/35[d]	23/35[d]
10/79	13/1100[d]	2/55	3/55	8/55[d]	8/55[d]
11/79	0/1200	0/60	0/60	7/60[d]	7/60[d]
12/79	3/500	0/25	2/25	2/75	3/25
1/80	0/700	0/35	0/35	0/35	0/35
2/80	0/700	0/35	0/35	0/35	0/35
3/80	0/1400	0/70	0/70	1/70	1/70
4/80	0/2100	0/105	0/105	0/105	0/105
5/80	130/1800[d]	9/90	9/90	10/90[d]	11/90[d]
6/80	160/1400[d]	5/70	5/70	5/70	8/70[d]
7/80	0/1400	0/70	2/70	2/70	3/70
8/80	0/700	0/35	3/35	3/35	3/35
Community B					
5/80	2/600	0/30	4/30[d]	4/30[d]	4/30[d]
6/80	5/1600	8/80	15/80[d]	16/80[d]	20/80[d]
7/80	0/600	0/25	0/25	0/25	0/25

[a] Community A practiced chlorination as the only treatment of drinking water. Community B used untreated ground water as the drinking water supply.

[b] Sample volumes of 100 ml were analyzed and typical colonies submitted to LTB and BGLB for verification. Thus, for 8/79, 3 verified coliforms were obtained from 4 samples.

[c] Number of completed positive presumptive tubes/total number tubes inoculated.

[d] Indicates occurrences in excess of allowable limit.

SECTION 5

DISCUSSION

Turbidity measurements are influenced by a number of suspension and instrument characteristics whose combined effect is difficult to predict (3,25). Turbidity may vary in its nature and composition from area to area, and since it is an optical property of a suspension, it will be influenced by particle size, shape, and number. Despite these limitations, turbidity measurements in drinking water are valuable indicators of water quality because clay-organic complexes at entry points to unfiltered distribution water may act as carriers for a variety of materials such as pesticides, heavy metals and bacteria (26,27). Similar to the coliform indicator concept, a high turbidity measurement is usually an indication of inadequate water quality. Turbidity determinations have advantages as indicators of water quality in that they are rapid, relatively inexpensive and can be performed continuously by in situ detectors.

The monitoring of coliform numbers during CLDMD determinations on waters with elevated turbidities and statistical analysis of these results have shown that turbidity interferes with proper chlorine disinfection of drinking water. This conclusion applies to surface water sources which are not filtered prior to disinfection. In the Pacific Northwest region, numerous public water supplies simply use disinfection as the only treatment even during periods of stormwater runoff. This results in elevated turbidity at both the raw water intake and at the consumers tap. The LFDC regression model (Model 3) predicts, assuming a constant chlorine dose, that an increase in turbidity from 1 NTU to 10 NTU in the surface water supply would result in a 8-fold decrease in efficiency of disinfection. This could imply that turbid drinking water (10 NTU) is 8 times more likely to carry pathogenic bacteria than a turbidity of 1 NTU. While several studies have previously demonstrated that the presence of particulate material in water interferes with disinfection, these studies have dealt primarily with virus inactivation or coliforms within nematodes and crustaceans (4,5,7,8). Since current practice utilizes coliforms as indicators of water potability, it is significant to note that naturally occurring turbidity at entry points to unfiltered distribution water interfered with disinfection of coliforms (Table 1). Our study included several different watersheds to demonstrate that results are probably applicable to most watersheds at least in the Pacific Northwest.

A mechanism for coliform survival at high NTU levels is possible if coliforms are imbedded in suspended particles and chlorine is not able

to come into contact with bacteria. Bacterial attachment to solids has been well documented (26,28,29,30). The increase in bacterial numbers as a result of blending chlorinated samples, and micrographs showing bacteria embedded in amorphous material provides further evidence that turbidity may protect bacteria from the action of chlorine.

The total organic carbon (TOC) associated with turbidity was primarily responsible for causing the chlorine demand of surface waters. These results are consistent with what is known about chlorine chemistry. Organic compounds are known to react with hypochlorite to form trihalomethanes (31). The presence of high TOC levels in raw water supplies produces two undesirable effects; it reacts with chlorine rendering it ineffective for disinfection, and the by-products of this reaction are trihalomethanes, some of which are known to be carcinogenic (32).

Turbidity and the associated TOC will adversely impact the maintenance of an effective free chlorine residual in a distribution system. The regression equation, chlorine demand = .40 + 0.086 (NTU), implies that a turbidity increase from 1 NTU to 6 NTU would result in a doubling of the chlorine demand. In this situation, assuming a constant chlorine dose, the chlorine demand of the raw water could remain unsatisfied and thus would interfere with the maintenance of a chlorine residual in the distribution system. With no free chlorine available, disinfection efficiency would also be impaired.

The statistical models can be used to predict the impact of turbidity in a general way on drinking water quality. Consider a raw water sample containing 400 total coliforms/100 ml which is chorinated with an initial dose of 1.0 mg/l chlorine (season = 12). If the turbidity of the raw water source is 1 NTU, a value of 1.2 mg/l total organic carbon (TOC) would be associated with the turbidity (Model 1). With this TOC value a chlorine demand of 0.45 mg/l could be predicted and result in a concentration of 0.55 mg/l free chlorine being available as a chlorine residual in the distribution system (Model 2). At 1 NTU, Model 3 indicates that a 460-fold decrease in coliform densities would result in the disinfected drinking water containing less than 1 coliform/100 ml. If a rainfall event resulted in the raw water increasing in turbidity from 1 NTU to 5 NTU, then 1.83 mg/l of TOC would be associated with the turbidity. This would result in a chlorine demand of 0.81 mg/l, in this example. If the chlorine dose remained constant, the residual after chlorination with 1.0 mg/l chlorine would be a 0.19 mg/l chlorine which is below the accepted limit for a desirable chlorine residual (1). A 180-fold reduction in coliform numbers would result in the 400 coliforms/100 ml initial count in raw water being reduced to 2 coliforms/100 ml in drinking water. This water would not meet the compliance regulation of not exceeding 1 coliform/100 ml as stated in the National Interim Primary Drinking Water Regulations (1). The above example demonstrates that turbidity can interfere with disinfection efficiency and the maintenance of a free chlorine residual.

The assessment of the bacterial quality of drinking water depends on the ability to make accurate microbiological determinations. The results indicated that turbidity interferred with the determination of coliform densities by the membrane filter technique. Both the incidence of masking and the magnitude of the interference with coliform detection increased as turbidity increased. Fryt (33) has observed that with increasing turbidity values over 1.8 NTU the differences between MF and MPN results tended to increase. Geldreich (11) has noted that more coliforms could be detected in waters containing turbidities of 1 to 5 NTU than in any other range of turbidities. Our results indicated that the magnitude of coliform masking increased from <1 coliform/100 ml in water samples of <5 NTU to >1 coliform/100 ml in water samples of >5 NTU. In addition, coliforms could be recovered from 26% of 56 distribution samples that apparently lacked coliforms by the standard MF technique. Coliforms that were isolated from masked coliform samples were identified as Citrobacter freundii and Enterobacter spp. These organisms have been previously reported to be the predominant species in chlorinated drinking water (34).

Several explanations may account for failure to recover typical coliforms by the MF technique: (i) coliforms may be entrapped in suspended materials and may not be able to outgrow to form colonies (9); (ii) high numbers of standard plate count bacteria accompanying elevated turbidities may be antagonistic to coliforms (10,11); or (iii) turbidity may alter the surface morphology of the membrane filters so that surface openings are not large enough to surround the entrapped bacteria, thus preventing optimum growth conditions (35). Coliform masking accompanying turbidity may be a combination of these factors.

In summary, turbidity is a useful indicator of potential problems in drinking water. Components of turbidity associated with TOC exert a continuous interference with disinfection efficiency, maintenance of a chlorine residual, and microbiological determinations. It is further concluded that turbidity control is justified to at least the 1 and 5 NTU levels to maintain adequate drinking water quality.

Interference with the detection of coliforms (masking) was found to occur at all steps in the S-MPN technique and will be a problem in any routine MPN coliform analysis of contaminated drinking water. The M-MPN technique doubled the incidence of coliform detection compared to that detected by the S-MPN technique.

The largest incidence of masking was recorded in the presumptive test. Coliform masking in presumptive media has been suggested by other studies (10,19) and was demonstrated quantitatively in the presumptive step of MPN analyses of seawater samples (36). However, parameters which influence the detection of coliforms from seawater (31,38) are probably not relevant to drinking water supplies (salinity, divalent cations). Indirect evidence for coliform interference was reported in a study of the bacteriological quality of 969 community drinking water supplies (10). In that study, the presence of non-coliform bacteria in

excess of 500 organisms/ ml correlated with a reduced frequency of coliform isolation from potable water samples. Geldreich et al. (10) speculated that competition by non-coliform bacteria for lactose and the presence of bacteria antagonistic to coliforms may result in coliform interference.

The detection of coliforms in the presumptive test is dependent on the fermentation of lactose with the production of gas in sufficient quantities so as to be visible in the Durham tube. Cultural conditions which influence either the activity or quantity of the enzymes responsible for gas production will ultimately influence the outcome of the presumptive tests. In this study, coliforms were isolated from negative presumptive tubes when the pH of the medium was acid (pH of 4.5) or slightly alkaline (pH of 7.2). These pH values have been reported as inhibitory to either the activity or inducibility of the formic hydrogenlyase enzyme system (39,40,41,42).

Another cause of the coliform masking in presumptive media may result from the injury of coliforms in the aquatic environment. The phenomenon of coliform injury has been shown to occur in surface waters (44) and chlorinated waters (45). One of the physiological affects of injury or stress was shown to be an increased lag time, compared to non-stressed cells, when inoculated into complex media (44). Since many bacteria are not as susceptible to stress as coliforms (46), non-coliform bacteria may have a reduced lag time when inoculated into lactose containing media. Therefore, non-coliform bacteria may be able to overgrow coliforms and produce conditions unsuitable for gas production.

Coliform masking in confirmatory MPN tests was also found to be a significant cause for the underestimation of coliforms in drinking water by the S-MPN procedure. The failure of coliforms to grow in BGLB when subcultured from lactose containing media has been well documented (19,20,47,48) and was extremely prevalent when chlorinated potable water samples were examined (48). Even though the suitability of BGLB as a confirmatory medium has been demonstrated (49,50,51), BGLB may not be the best medium to use in the confirmatory test when chlorinated drinking water or untreated surface water supplies are examined.

False-negative completed tests in the S-MPN technique occurred at a lower frequency than the false-negative presumptive or confirmed tests. This was evident in both types of water supplies examined. Coliform masking in the completed step may be a function of the inhibitory nature of EMB agar or overgrowth by non-coliform bacteria on the agar medium obscuring typical colonies. The use of the multiple inoculation procedure and the use of LB and LTB, where an isolate was checked for the ability to ferment lactose, recovered significantly more lactose fermenting isolates than did the technique recommended by Standard Methods.

Masking of the various coliform genera in the S-MPN procedure did not appear to be a simple function of their relative numbers in the water sample. Different susceptibilities of the specific coliform

bacteria to the inhibitory nature of the media used in the MPN technique, to competition for lactose by non-coliform bacteria, and to antagonistic products produced by non-coliform bacteria, may result in certain coliforms being more prone to masking than others. Therefore, the probability of recovering a specific coliform genus from a masked MPN test is probably a function of both the number relative to other coliform species and the ability to grow and produce gas in presumptive and confirmatory media.

The use of the M-MPN procedure has shown that both the S-MPN and MF techniques failed to detect as well as underestimated the number of coliforms in potable drinking water. Therefore, the use of standard techniques in their present form may lead to situations where the drinking water supply is considered potable, yet a health hazard or potable water treatment deficiency may exist. Failure of the standard techniques to recover coliforms, when indeed they are present, as illustrated here and elsewhere (17,18,52) may be one of the important factors leading some to criticize the indicator organism concept (53).

Results obtained in the current study indicated the M-MPN procedure was a more sensitive coliform detection technique than either of the standard techniques. Since the M-MPN technique was a labor and material intensive procedure, it could not be applied on a routine basis. Therefore, an abbreviated form of the M-MPN technique was developed and compared to the S-MPN and MF techniques for coliform recovery from water samples. This alternative MPN technique was found to be superior to the S-MPN and MF technique in both the detection as well as enumeration of coliforms from drinking water samples.

The use of the MI procedure yielded greater numbers of coliforms and a greater number of coliform contaminated drinking water samples than the IC procedure in the completed test of the A-MPN technique. Examination of gas-positive secondary tubes of LTB which were inoculated by the MI procedure indicated a coliform in pure culture could always be obtained by streaking onto M-Endo agar LES.

A comparision of inoculation techniques and secondary lactose containing media in the A-MPN indicated the IC procedure with both LB and LTB or the MI procedure with LTB yielded comparable numbers of coliforms for both types of water samples examined. Therefore, the IC procedure employing LB and LTB is an alternative to the MI procedure but is more time consuming and requires the use of two secondary media.

Additional studies need to be conducted to determine both the efficiency of the A-MPN technique relative to standard MF and MPN techniques and the efficacy of using the MI procedure in the completed test of the A-MPN technique. Such studies should include the examination of contaminated drinking water samples obtained from different geographic locations.

The enhanced sensitivity of the A-MPN technique was due to the increased detection of certain coliform genera. For untreated surface water; Citrobacter, Enterobacter, Escherichia, Hafnia, and Yersinia and

for drinking water samples; Citrobacter, Enterobacter and Klebsiella were isolated more frequently by the A-MPN technique than by the S-MPN technique.

The significance of the increased sensitivity of the A-MPN technique due to the isolation of Citrobacter and Enterobacter may be open to question. Although Citrobacter and Enterobacter species may be present in water supplies as a result of non-fecal contamination from such sources as soil or plants, these coliforms could arise from either raw or secondary sewage (54,55). Citrobacter may be present in human fecal material at 10^5 to 10^6 cells per gram (55). Therefore, the presence of Citrobacter or Enterobacter in drinking water could indicate contamination of sewage origin and their presence should be regarded as a possible indication of a significant health risk.

The use of the A-MPN technique in the routine monitoring of drinking water supplies resulted in increased numbers of coliforms being detected over the standard MF and MPN techniques. As a consequence, compliance with the Safe Drinking Water Act regulations of the two communities were affected. If the A-MPN technique was adopted as an official method and used for routine monitoring, the improved coliform detection would provide a more sensitive assessment of water treatment efficiency. In addition, increased protection would be provided to the consumer due to the detection of low level contaminant releases which might contain some pathogen component.

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